conjunction with elevated SR Ca^{2+} load to generate spontaneous Ca^{2+} waves in rabbit cardiomyocytes.

3040-Pos Board B145

Regulation of Sarcoplasmic Reticulum [Ca^{2+}] during Rest in Rabbit Ventricular Myocytes

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During diastole, ryanodine receptor Ca^{2+} release channels are not completely quiescent, thus providing a pathway for significant sarcoplasmic reticulum (SR) Ca^{2+} leak. Cytosolic Ca^{2+} can be pumped back into the SR by the SR Ca^{2+}-ATPase (SERCA) or extruded by Na^{+}-Ca^{2+} exchanger (NCX). Therefore, the activity of Ca^{2+} transport systems during diastole plays a critical role in setting SR Ca^{2+} load under normal conditions and in disease states. Using confocal microscopy, we studied mechanisms that control intra-SR free Ca^{2+} ($[Ca^{2+}]_{SR}$) at rest in rabbit ventricular myocytes. We compared the rate of $[Ca^{2+}]_{SR}$ decline (with Fluo-5N) after rest from electrical pacing in control conditions and after SERCA inhibition with thapsigargin (TG; 10 μM). We found that the rate of $[Ca^{2+}]_{SR}$ decline increased only ~30% after SERCA blockade compared to control conditions (from 10.9 in control to 14.1 μM/s in the presence of TG). Similar results were obtained by measuring the rate of decline of total SR Ca^{2+} content, estimated from caffeine-induced Ca^{2+} transient amplitude (with Fluo-4). Inhibition of NCX by Ni^{2+} (5 mM) or by 0 $[Na^{+}]$/0$[Ca^{2+}]$ solution significantly slowed $[Ca^{2+}]_{SR}$ decline during rest (by 3.4 times), but did not prevent it. Simultaneous inhibition of NCX with 0 $[Na^{+}]$/0$[Ca^{2+}]$ solution and plasmalemmal Ca^{2+} ATPase with La^{3+} (1 mM) completely prevented $[Ca^{2+}]_{SR}$ decline during rest. These results indicate that in rabbit ventricular myocytes the predominant mechanism for cytosolic Ca^{2+} removal during rest is NCX but not SERCA-mediated Ca^{2+} uptake. These data are compatible with a model in which the majority of SR Ca^{2+} leak occurs through clusters of ryanodine receptors in the junctional SR that closely oppose NCX in the dyadic cleft.

3041-Pos Board B146

Increased Myofilament Ca^{2+} Sensitivity Decreases Sarcomere Length and Increases Spark-Spark Interactions

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People with familial hypertrophic cardiomyopathy (FHC) harboring mutations of cardiac troponin T (cTnT) are often at a high risk of sudden cardiac death. Transgenic mice harboring some of these cTnT mutations show increased myofilament sensitivity to Ca^{2+} and also shortened diastolic sarcomere length (SL). Our computational studies predicted that decreasing the distances between Ca^{2+} release units (CRUs) of the sarcoplasmic reticulum (SR) by decreasing SL can destabilize the Ca^{2+} control system and increase the probability of spontaneous Ca^{2+} waves. Destabilization results from enhanced crosstalk between neighboring CRUs. In this study we mimic the greater myofilament Ca^{2+} sensitivity conferred by cTnT mutations using the myofilament Ca^{2+} sensitizer EMD 57033 (EMD). At concentrations up to 3 μM, EMD had no effect on either the peak Ca^{2+} transient or the diastolic Ca^{2+} levels and did not alter the SR Ca^{2+} load. To test the prediction that SL shortening increases the coupling between CRUs, we loaded myocytes with Di8-ANEPPS and Fluo-4 and simultaneously measured SL and Ca^{2+} sparks in 2 spatial dimensions using the Zeiss 5 Live high-speed 2-D scanning confocal microscope. EMD (1.5 μM) decreased SL significantly compared to the control cells in normal Tyrode (1.58 μm vs.1.69 μm, $p<0.05$). The spark coupling strength measures the influence of one CRU on another and is derived from an analysis of the spatiotemporal distribution of Ca^{2+} sparks. EMD treatment significantly increased the spark coupling strength 2.5 fold. The enhanced spark-spark coupling as diastolic SL decreases may contribute to increased frequency of spontaneous Ca^{2+} waves during diastole that can lead to triggered arrhythmias and sudden cardiac death in FHC.

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RyR2 Channel Activity Determines the Potency of State-Dependent RyR2 Blockers for Suppressing Arrhythmogenic Calcium Waves

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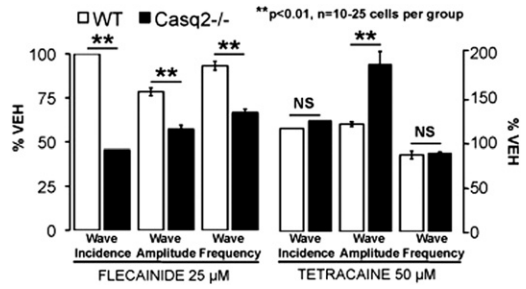
Mutations in ryanodine receptors (RyR2) or calsequestrin (casq2) cause catecholaminergic- polymorphic ventricular tachycardia (CPVT). We previously reported that the RyR2 open-channel blocker flecainide (FLEC) suppresses Ca2+ waves and prevents CPVT in mice and humans. Here we test the hypothesis that the open-state block by FLEC significantly contributes to FLEC efficacy in CPVT. We reasoned that FLEC would preferentially affect myocytes lacking casq2 (casq2-/-), which have higher rates of spontaneous RyR2 channel openings compared to WT channels. To test this hypothesis, we compared FLEC with tetracaine, a RyR2 channel blocker that has no state dependence and binds equally well to closed RyR2 channels. We found that FLEC reduced the incidence, amplitude and frequency of Ca2+ waves with significantly higher potency in casq2-/- myocytes compared to WT myocytes (Figure). In contrast, tetracaine did not suppress Ca2+ waves and had equal potency in WT and casq2-/- myocytes (Figure). Conclusion: RyR2 channel activity likely determines the potency of open-state RyR2 blockers such as FLEC for suppressing arrhythmogenic Ca2+ waves, a mechanism likely relevant to FLEC antiarrhythmic efficacy in CPVT. NIH HL88635 & HL71670.



3043-Pos Board B148

FKBP12.6 'Stabilises' Cardiac SR Ca^{2+}-Release by Antagonising High-Affinity Reversible Activation of RyR2 by FKBP12

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FKBP12.6 is thought to play an important cardioprotective role, however, the underlying mechanism is not understood. Since FKBP12 is structurally similar to FKBP12.6 but is found at much higher levels (1-3 μM), we investigated the effects of both FKBP12 and FKBP12.6 on RyR2 single-channel function and on SR Ca^{2+}-release in rat isolated permeabilised cardiomyocytes. FKBP12 increased RyR2 open probability (Po) in a concentration-dependent, reversible manner (EC_{50} 51 nM). Physiological levels of FKBP12 (3 μM) increased Po from 0.187 ± 0.051 to 0.657 ± 0.111 (SEM; n=14; $P<0.001$). FKBP12.6 (200 nM), itself, did not significantly alter RyR2 Po, but was a very effective antagonist of FKBP12, shifting the FKBP12 EC_{50} to 4 μM. In permeabilised myocytes perfused with Fluo-5F, spontaneous waves of Ca^{2+}-induced Ca^{2+}-release were induced by 234 nM Ca^{2+} in the mock cytosolic solution. Perfusion with FKBP12 (3 μM) increased wave frequency from 0.34 ± 0.04 Hz to 0.52 ± 0.07 Hz (SEM; n=14; $p<0.03$). 10 mM caffeine produced a larger Ca^{2+}-transient in control (2.21 ± 0.11; F/Fo) than in FKBP12 (1.47 ± 0.16; n=6; $p<0.003$) indicating lower SR Ca^{2+}-content. Perfusion with FKBP12.6 (200 nM) alone, had no significant effect yet it reduced the ability of FKBP12 to increase wave frequency (49.9 ± 5.8% increase over control in the absence of FKBP12.6 vs. 16.2 ± 2.1% in the presence). Our single-channel experiments demonstrate that FKBP12 is a high affinity, potent activator of RyR2. FKBP12.6 acts as an antagonist of FKBP12 at RyR2 but itself possesses minimal efficacy. Our cellular experiments suggest that this is the underlying mechanism by which FKBP12.6 acts to 'stabilise' or reduce SR Ca^{2+}-release in cardiac cells. Thus, the balance between the opposing actions of FKBP12 and FKBP12.6 on RyR2 gating may be crucial for normal EC-coupling in cardiac cells.

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Increased Levels of Micrornas miR-1 and miR-133 in Failing Heart Underlie Dissociation of Phosphatase Activity from RyR2 Complex Resulting in Enhanced RyR2 CaMKII-Dependent Phosphorylation and Cardiac Arrhythmias

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Increased propensity of ventricular myocytes to arrhythmogenic spontaneous SR Ca release and afterdepolarizations in heart failure (HF) has been linked to abnormally high activity of RyR2. Growing evidence supports hyperphosphorylation of RyR2 at the CaMKII site S-2814 as a potential mechanism for altered RyR2 function. However, the specific molecular mechanisms underlying RyR2 hyperphosphorylation remain poorly understood.

MicroRNAs are small noncoding RNAs that regulate protein expression by interfering with mRNAs of target genes. We recently reported that 2-fold overexpression of microRNA miR-1 enhances CaMKII-dependent RyR2 phosphorylation by disrupting protein phosphatase 2A scaffolding to the RyR2, resulting in increased activity of the channel and Ca-dependent afterdepolarizations in myocytes. In the present study, we used a canine model of nonischemic HF to test the hypothesis that the HF-related alterations in RyR2 phosphorylation levels are caused by a decrease in phosphatase activity localized to RyR2 due to enhanced expression of two most abundant muscle-specific microRNAs miR-1 and miR-133. qRT-PCR studies revealed that the